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Stereoselective inhibition of glutamate carboxypeptidase by organophosphorus derivatives of glutamic acid

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Abstract—A series of alkyl and aryl phosphonyl, thiophosphonyl, and dithiophosphonyl derivatives of (*S*)- and (*R*)-glutamic acid were prepared and examined for inhibitory potency against glutamate carboxypeptidase (carboxypeptidase G). The acquisition of the phosphonamidodithioic acids and the individual phosphonamidothioic acid diastereomers was achieved through a common phosphonamidothiolate precursor, which also allowed for the chromatographic resolution of the chiral phosphorus center of the phosphonamidothioic acids. The most potent inhibitor of the series was the *n*-butylphosphonamidate derivative of the natural isomer of glutamic acid. Although each diastereomeric pair of three phosphonamidothionates exhibited stereoselective inhibition consistent with the configuration of the chiral phosphorus center, this effect was generally not remarkable. More important, was the effect of carbon stereochemistry upon glutamate carboxypeptidase inhibition as exemplified by a limited series of enantiomeric pairs of phosphonamidate and phosphonamidodithionate derivatives of glutamic acid. The phosphonamidate analogs derived from the unnatural stereoisomer of glutamic acid were devoid of inhibitory potency in contrast to their enantiomers. Surprisingly, the phosphonamidodithionates derived from the unnatural stereoisomer of glutamic acid demonstrated greater inhibitory potency than their naturally-derived antipodes.

1. Introduction

Our research efforts have been recently aimed at developing potent competitive inhibitors for glutamate carboxypeptidases. This class of enzymes encompasses various metallopeptidases such as *N*-acetylated-alpha-linked-acidic dipeptidase (NAALADase),[1] prostate-specific membrane antigen (PSMA),[2] pteroylpoly-glutamate hydrolase (PPH),[3] and carboxypeptidase G (CPG).[4] The acquisition of inhibitors for such enzymes is expected to further the understanding of the biological role of these metallocarboxypeptidases as well as to serve in the elucidation of germane active-site features. In addition, inhibitors of carboxypeptidase G_2 have been recently sought for use in inhibiting non-tumor-localized enzymes in ADEPT strategies.[5]

Based upon preliminary evidence, alkylphosphonyl and alkylthiophosphonyl derivatives of glutamic and 2-hydroxyglutaric acid have exhibited promise as potent tetrahedral-intermediate analog inhibitors of glutamate-liberating metallopeptidases.[6] Although the phosphonyl motif often provides suitable inhibitory potency toward metallopeptidases, thiophosphonyl analogs maintain the unique potential for probing enzyme active-site architecture with complementary chiral phosphorus centers. In some cases, a single alkylthiophosphonyl stereoisomer displayed inhibitory potency notably greater than either a stereoisomer of antipodal phosphorus stereochemistry or a respective alkylphosphonyl analog.[6b] The basis for the enhanced inhibitory potency of such compounds, especially against zinc-metallopeptidases, was presumably due to favorable zinc–sulfur interactions within enzyme active sites.[7]

Keywords: Phosphonamidothionate; Phosphonamidodithionate; Stereoselective; Metallopeptidase.
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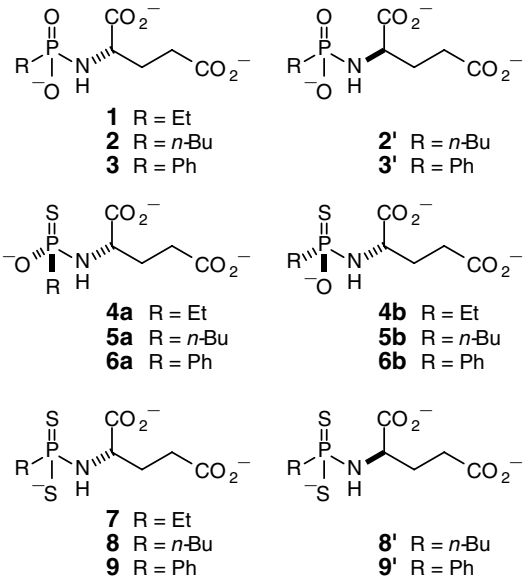


Figure 1.

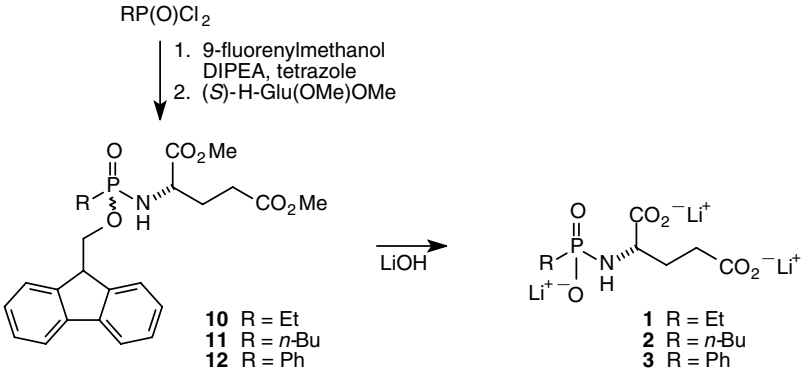
The focus of the present study was threefold. First, we were interested in exploring the significance of phosphorus stereochemistry of thiophosphonyl derivatives of glutamic acid upon the inhibition of glutamate carboxypeptidase. Second, we wanted to determine if dithiophosphonyl analogs could serve as reasonably potent metalloprotease inhibitors. Lastly, we wanted to assess the significance of glutamic acid's α-carbon stereochemistry upon the inhibitory potency of glutamate carboxypeptidases by phosphonyl and dithiophosphonyl derivatives of glutamic acid.

The compounds for which the synthesis and inhibitory potency against glutamate carboxypeptidase are described herein are shown in Figure 1. Prime designations for both phosphonamidates **2** and **3** and phosphonamidodithionates **8** and **9** identify these compounds as possessing unnatural or (*R*)-stereochemistry at the alpha carbon of gluatmic acid. All other compounds listed without prime designations are derivatives of (*S*)-glutamic acid. Compounds designated as '**a**' isomers maintain a (*R*)-configuration at phosphorus while those designated as '**b**' possess antipodal configuration at that center.

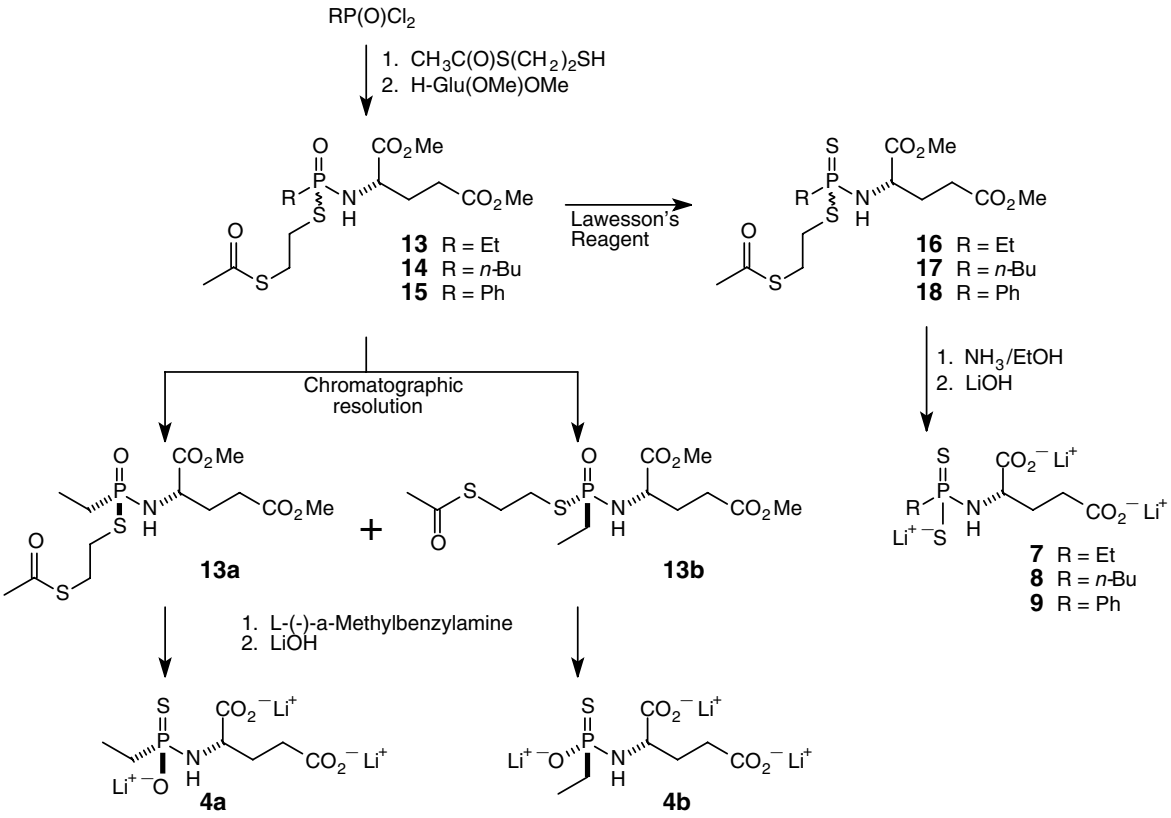
2. Results and discussion

Although phosphonamidate **3**, in which the natural isomer of glutamic acid was incorporated, was available from previous studies,[6c] we prepared additional material along with analogs **1** and **2** as described in Scheme 1. Starting with the appropriate phosphonic dichloride, intermediate phosphonamidates **10**–**12** were prepared and subsequently deprotected to provide the desired phosphonamidoic acids **1**–**3**. In previous studies, we found the fluorenylmethoxy group to be a convenient protecting ligand for the preparation of phosphonic acid derivatives and one that can be concomitantly removed with methyl esters under mild basic conditions.[8,9] The enantiomeric analogs of **1**–**3**, namely **1′**–**3′**, in which the configuration of the α-carbon on glutamic acid was (*R*), were prepared in the manner described by Scheme 1 with the exception that (*R*)-glutamic acid dimethyl ester was used in the latter half of the first step. To confirm that inhibitors **1**–**3** would be sufficiently stable under the conditions of the enzyme inhibition studies, stability studies at pH 7.3 were conducted. No decomposition of phosphonamidates was observed under the these conditions over a 15 h period.

The individual phosphonamidothioic acid stereoisomers **5a** and **5b** as well as **6a** and **6b** were available from previous work in our lab.[8] The stereoisomers of the ethyl analog (**4a** and **4b**) were prepared as outlined in Scheme 2. Following the chromatographic resolution of phosphonamidothionate intermediates **13a** and **13b**, deprotection of the sulfur ligand was accomplished stereospecifically with methylbenzylamine. Initially it was envisioned that typical deprotection of the β-(acetylmercapto)ethyl protecting group on sulfur by a hindered base such as quinine would yield a salt amenable to purification by crystallization. Though this technique has been successful for other analogs,[8] the reaction of one isomer (**13a**) with quinine or strychnine resulted in an impure salt that was recalcitrant to attempts at recrystallization. However, it was found that reaction of **13a** and **13b** each with L-(−)-α-methylbenzyl-



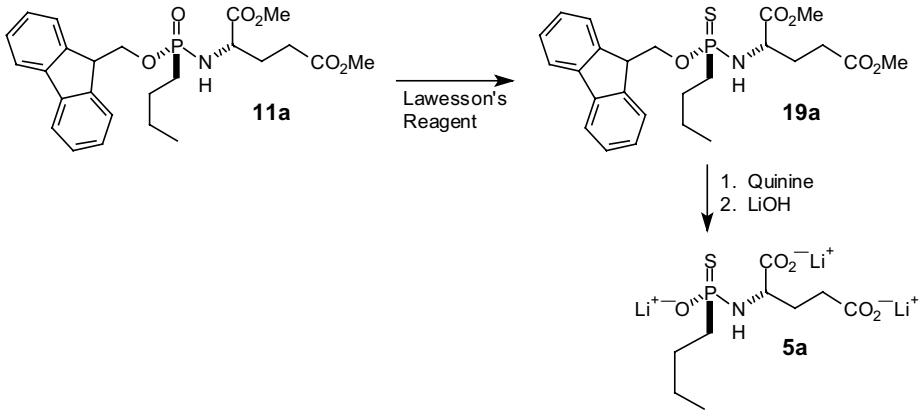
Scheme 1.



Scheme 2.

amine[10] was stereospecific leading to pure deprotected products. Subsequent hydrolysis of the methyl esters with LiOH afforded **4a** and **4b**, respectively.

We had previously reported that through fractional crystallization, the individual stereoisomers of intermediate **11** possessing antipodal phosphorus stereochemistry (**11a** and **11b**) could be obtained.[8] Furthermore, the individual stereoisomers **5a** and **5b** were obtained through a sequence of stereospecific thionation and deprotection steps not involving the chiral phosphorus center (Scheme 3).[8] Diastereomer **11a** was crucial in establishing the absolute configuration of the central phosphorus atom of inhibitors **4**–**6**. During the course of this investigation, a crystal of phosphonamidate **11a** suitable for X-ray crystallography was identified. This crystal was available from our previous work aimed at resolving the individual stereoisomers of **11a** through fractional crystallization.[8] The resulting X-ray crystallographic analysis established the stereochemical configuration of the phosphorus center in **11a** as illustrated in Figure 2.[11] With that known, as well as the stereospecificity of the subsequent chemical reactions in Scheme 3 performed previously,[8] we were able to unambiguously determine the absolute configuration of the phosphorus center in inhibitor **5a**. Briefly, phosphonamidate **11a** was first stereospecifically thionated with Lawesson's reagent.[12] The final deprotection steps with quinine



Scheme 3.

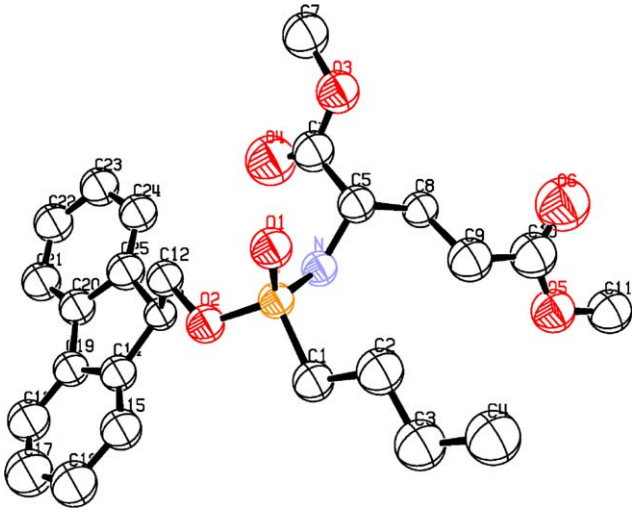


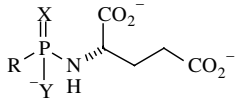
Figure 2. X-ray crystal structure of phosphonamidate **11a**.

and LiOH, reactions which did not involve the chiral phosphorus center, provided **5a**. By comparing the ^{31}P NMR chemical shift of this material with that of isomers **5a** and **5b** prepared previously via a route analogous to that outlined in Scheme 2, the phosphorus stereochemistry of both **5a** and **5b** was established.[8] Numerous unsuccessful attempts aimed at preparing suitable crystals of the remaining diastereomeric phosphonamidothionate pairs (**4a**,**b** and **6a**,**b**) as well as their synthetic intermediates were made. Therefore, the absolute configurational assignments at the phosphorus atom in these diastereomeric pairs were surmised by comparing trends in the ^{31}P NMR chemical shifts and rates of desulfurization in buffer with those of **5a** and **5b**.

Stability studies were conducted to confirm that inhibitors **4–6** would be sufficiently stable under the conditions of the enzyme inhibition studies. The impetus for such studies was based upon earlier reports that demonstrated desulfurization of phosphonamidothionates occurred through acid-catalyzed hydrolysis via a cyclic mixed phosphonyl–carbonyl anhydride intermediate, resulting from direct attack of the α-carboxyl group on phosphorus.[13] Indeed, desulfurization of phosphonamidothionates **4–6** at pH 7.3 to the corresponding phosphonamidates **1–3** was observed by ^{31}P NMR (Table 1). With respect to the individual stereoisomers of phosphonamidothionates **4–6**, it was noted that for each diastereomeric pair, the isomers exhibiting a greater ^{31}P NMR chemical shift was consistently less stable to desulfurization at pH 7.3 than the respective upfield isomers. We hypothesized that the stereospecific formation of the purported cyclic mixed phosphonyl–carbonyl anhydride intermediates[13] during desulfurization was more energetically favorable for one diastereomer. Accordingly, it would be expected that the two diastereomers would exhibit, respectively, different rates of desulfurization. These results indicated that the more stable stereoisomers of each diastereomeric pair of **4–6** more likely than not possessed the same stereochemical configuration at phosphorus while those less stable stereoisomers maintained the opposite stereochemical configuration. With the phosphorus stereochemistry of **5a** and established as described above, the analogous downfield stereoisomers **4a** and **6a** were thus presumed to also possess an (*R*)-configuration at the chiral phosphorus center while the respective diastereomers **4b**, **5b**, and **6b** were presumed to maintain the opposite configuration (S_P)[14] at phosphorus (Table 1).

Phosphonamidodithionates **7–9** were prepared as outlined in Scheme 2. Following the preparation of the fully protected phosphonamidothionates **13–15**,[8] which were each obtained as mixture of diastereomers unresolved at phosphorus, thionation with Lawesson's reagent provided **16–18** as immediate precursors to the target phosphonamidodithionates **7–9**. Removal of the protecting group from the sulfur ligand to phosphorus with methanolic ammonia followed by deprotection of the methyl esters with LiOH provided phosphonamidodithionates

Table 1. The effect of phosphorus ligands and stereochemistry upon the inhibition of glutamate carboxypeptidase



Entry	R	X	Y	P-configuration	^{31}P NMR[a]	$t_{1/2}$ (min)[b]	K_i (μM)[c]
1	Et	O	O	—	29.12	—	63 (1.1)
4a	Et	S	O	*R*	71.01	24.5	2.2 (0.13)
4b	Et	S	O	*S*	69.56	49.9	20 (1.6)
7	Et	S	S	—	90.54	—	25 (1.3)
2	*n*-Bu	O	O	—	27.87	—	0.63 (0.15)
5a	*n*-Bu	S	O	*R*	69.31	36.5	1.2 (0.11)
5b	*n*-Bu	S	O	*S*	69.01	66.9	3.7 (0.17)
8	*n*-Bu	S	S	—	90.11	—	22 (1.4)
3	Ph	O	O	—	15.24	—	24 (1.2)
6a	Ph	S	O	*R*	57.28	23.0	13 (0.65)
6b	Ph	S	O	*S*	56.29	40.5	46 (2.0)
9	Ph	S	S	—	81.36	—	530 (71)

[a] Chemical shifts externally referenced to 85% H_3PO_4 (δ = 0.00 ppm).
[b] No detectable degradation was observed for **1–3** and **7–9**.
[c] Standard deviation in parentheses.

7–**9** in good yield. It was noted that using a higher concentration of methanolic ammonia (7 N vs 2 M) and in larger excess (30 equiv vs 10 equiv) in the first step of the deprotection resulted in a more complete reaction and minimized the appearance of side products. The enantiomeric analogs of **8** and **9**, namely **8′** and **9′**, in which the configuration of the α-carbon on glutamic acid was (*R*), were also prepared in the manner described by Scheme 2 with the exception that (*R*)-glutamic acid dimethyl ester was used in the latter half of the first step for the formation of **14′** and **15′**. In stability studies at pH 7.3, no notable decomposition of the phosphonamidodithionates **7**–**9** was observed.

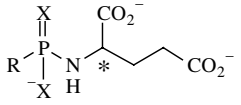
In addition to characterization by ^{1}H and ^{13}C NMR as well as high-resolution mass spectrometry, each target compound (**1**–**9**) displayed a single resonance in the ^{31}P NMR spectrum indicating complete deprotection in the last step and formation of clean product. Once obtained in sufficient quantity, the individual inhibitors **1**–**9** were examined for inhibitory potency against glutamate carboxypeptidase employing an HPLC-based assay to conventionally measure enzymatic hydrolysis of methotrexate. With K_m and V_{MAX} previously determined (1.6 μM and 25 μmol min^{-1} mg-protein^{-1}, respectively),[15] Dixon analyses were performed to obtain K_i values the inhibitors (Tables 1 and 2). In all analyses, the initial substrate (methotrexate) concentration was 10 μM and triplicate determinations were made and averaged for each inhibitor concentration, which generally varied within a range from 5 to 200 μM, depending upon the potency of the individual inhibitors. The correlation coefficient of the Dixon analyses for each inhibitor was greater than 0.99.[16] For each analogous set (R = Et, *n*-Bu, Ph) of organophosphorus derivatives of the natural isomer of glutamic acid (Table 1) the most consistently observed trend in the inhibitory potency against glutamate carboxypeptidase was held by the diastereomeric pairs of phosphonothionates **4**–**6**. In each case the (R_P)-isomer was a more potent inhibitor than the (S_P)-isomer. The relatively unique inhibitory potency of the **5a** and **5b** suggests that butyl ligand may achieve greatest binding to the enzyme, presumably through hydrophobic interactions, which are not as complete nor as accessible for the ethyl or phenyl ligands of **4a**,**b** and **6a**,**b**, respectively. Although the phosphonothioic acid stereochemistry has the potential to be significant with respect to metallopeptidase inhibition, in some cases (**5a/b** and **6a/b**) we observed that it was relatively independent with K_i values being only two- to three-fold different for diastereomeric pairs.

The phosphonamidodithionates **7**–**9** were consistently less potent inhibitors than either of their respective diastereomeric analogs **4a**–**6b**, with the enzyme apparently discriminating the least for the smallest alkyl analogs (**4a**,**b**, and **7**). It was hypothesized that the loss of inhibitory activity against glutamate carboxypeptidase as a result of the replacement of the oxygen ligand of the phosphonamidothionates **4**–**6** with sulfur (**7**–**9**), as had been similarly postulated previously for inhibitors of carboxypeptidase A, was due to the considerably greater length of the P–S bond (1.85 Å) compared to that of the P–O bond (1.39 Å).[17] Hence, the difference in bond length between P–S and P–O could cause sufficient steric congestion in the vicinity of an active-site zinc ion in a metalloprotease resulting in weaker enzyme–inhibitor interactions. Not surprising, in the case where the steric bulk contributions by the alkyl ligand to phosphorus was less significant, specifically when R = Et, the effect of such increased steric congestion due to the two sulfur ligands of the phosphonamidodithionates was observed to be less dramatic.

When the inhibitory potencies of phosphonamidates **1**–**3** were compared to their respective phosphonamidothionate analogs **4**–**6**, a different relationship was observed in each case. While the *n*-butylphosphonamidate **2** was more potent than both phosphonamidothionate diastereomers **5a** and **5b** and the contrary was true for the ethyl analogs **1**, **4a**, and **4b**. The phenylphosphonamidate **3** exhibited intermediate inhibitory potency when compared to both phosphonamidothionates **6a** and **6b**. As such, no simple trend could be identified for the comparison of phosphonamidates with the corresponding phosphonamidothionates.

In order to determine if the stereochemistry of the α-carbon on glutamic acid could attenuate the inhibitory potency of the phosphorus moiety, the inhibition of glutamate carboxypeptidase by phosphonamidates **2** and **3** and phosphonamidodithionates **8** and **9** were

Table 2. The effects of α-carbon stereochemistry upon the inhibition of glutamate carboxypeptidase



Entry	R	X	Y	C-configuration	K_i (μM)[a]
2	*n*-Bu	O	O	*S*	63 (1.1)
2′	*n*-Bu	O	O	*R*	ND
8	*n*-Bu	S	S	*S*	22 (1.4)
8′	*n*-Bu	S	S	*R*	14 (3.2)
3	Ph	O	O	*S*	24 (1.2)
3′	Ph	O	O	*R*	ND
9	Ph	S	S	*S*	530 (71)
9′	Ph	S	S	*R*	77 (5.0)

[a] ND = no detectable inhibition at 500 μM inhibitor. Standard deviation in parentheses.

compared with their enantiomers **2′**, **3′**, **8′**, and **9′**, respectively. When the configuration of glutamate was inverted as in the phosphonamidates **2′** and **3′**, a complete loss of inhibitory potency was observed. Assuming that the glutamate carboxylates served to anchor the inhibitor into the binding site, these results suggested that inverting stereochemistry at the α-carbon could prevent the inhibitors **2′** and **3′** from adopting a conformation in which the phosphonyl oxygens could interact sufficiently with an active-site zinc ion. Conversely and surprisingly, the unnatural glutamate-containing phosphonamidodithionates **8′** and **9′** proved to be more potent inhibitors than their respective naturally-derived enantiomers **8** and **9**. For the phosphonamidodithionates **8** and **9**, steric crowding in the active site due to the two sulfur ligands was hypothesized to be responsible for the exhibited reduced inhibitory potency when compared to analogous phosphonamidothionates (**5** and **6**) and phosphonamidates **2** and **3**. Thus it was postulated that by inverting the stereochemistry of the glutamate α-carbon of phosphonamidodithionates **8** and **9**, their enantiomers **8′** and **9′** were able to adopt conformations in which the dithiophosphonyl moiety imparted less steric congestion in the active site than **8′** and **9′** and that inhibitory potency could be recovered.

3. Conclusion

In summary, the most potent inhibitor of glutamate carboxypeptidase identified in this study, as determined by K_i values, was phosphonamidate **2** in which the phosphonyl moiety possessed the natural isomer of glutamic acid and the *n*-butyl group as the remaining ligands to phosphorus. For the diastereomeric pairs of phosphonamidothionates **4**–**6**, the least stable inhibitor of each pair (**4a**, **5a**, **6a**), presumably possessing *R*-configuration at phosphorus, consistently exhibited greater stereoselective inhibition than its diastereomer. Although the natural stereochemistry of glutamic acid was presumed to be required for effective inhibitor binding to the active site of glutamate carboxypeptidase, analogs derived from the unnatural amino acid exhibited a surprisingly improved inhibitory potency only when they possessed a phosphorus moiety of greater steric bulk as was the case with phosphonamidodithionates **7′**–**9′**. It is expected that these results will be of value in the consideration of inhibitor designs for other glutamate carboxypeptidases of medical importance such as PSMA and NAALADase.

4. Experimental

4.1. Synthesis

4.1.1. General. All solvents used in reactions and diisopropylethylamine (DIPEA) were either obtained anhydrous or freshly distilled prior to use. All other reagents were used as supplied unless otherwise stated. Liquid (flash) chromatography was carried out using silica gel 60 (230–400 mesh). ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker DRX 300 MHz NMR spectrometer. ^{1}H NMR chemical shifts are relative to TMS (δ = 0.00 ppm), $CDCl_3$ (δ = 7.26 ppm), or CD_3OD (δ = 4.87 and 3.31 ppm). ^{13}C NMR chemical shifts are relative to CD_3OD (δ = 49.15 ppm) or $CDCl_3$ (δ = 77.23 ppm). ^{31}P NMR chemical shifts in $CDCl_3$, CD_3OD, or D_2O were externally referenced to 85% H_3PO_4 (δ = 0.00 ppm) in $CDCl_3$, CD_3OD, and D_2O, respectively. Combustion analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. High-resolution mass spectra (FAB) were performed by the University of Notre Dame Mass Spectrometry Facility, Notre Dame, IN 46556–5670. X-ray crystal analysis was performed by Dr. Stephen Pavkovic, Loyola University of Chicago, Chicago, IL and the crystal structure of **11a** was deposited at the Cambridge Crystallographic Data Centre (deposition number CCDC 200063). Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 200063. Copies if the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

4.1.2. General procedure for phosphonamidates 10–12. A solution of 9-flourenemethanol (2.38 mmol, 0.467 g) and DIPEA (2.38 mmol, 0.308 g) in benzene (8.0 mL) was added dropwise to a stirred solution of the appropriate alkyl or arylphosphonic dichloride (2.61 mmol), 1*H*-tetrazole (0.47 mmol, 0.033 g), and benzene (8.0 mL) under an $Ar_{(g)}$ atmosphere at 4 °C. The solution was stirred for 3 h during which it warmed to room temperature. A solution of neutralized L-glutamic acid dimethyl ester (2.85 mmol, 0.499 g), DIPEA (2.85 mmol, 0.368 g), and benzene (3.5 mL) was added and the reaction mixture was stirred for an additional 3 h, filtered, and concentrated in vacuo to a yellow oil. The products were purified by flash chromatography, to give white solids.

4.1.3. *N*-[9-Fluorenylmethoxy(ethyl)phosphinyl]-L-glutamic acid dimethyl ester (10). Chromatography conditions: ethyl acetate/hexane 3:1 v:v, R_f = 0.14. Yield: 27%. ^{1}H NMR (acetone-d_6): δ 1.05 (dt, J = 11.1, 4.5 Hz, 3H), 1.63–1.90 (m, 4H), 2.39–2.46 (m, 2H), 3.55 and 3.57 (s, 3H), 3.63 and 3.64 (s, 3H), 3.94–4.31 (m, 4H), 7.27–7.41 (m, 4H), 7.69 (d, J = 7.2 Hz, 2H), 7.83 (d, J = 7.2 Hz, 2H). ^{31}P NMR ($CDCl_3$): δ 36.85, 37.49. Anal. Calcd for $C_{23}H_{28}NO_6P$: C, 62.0; H, 6.3; N, 3.1. Found: C, 61.67; H, 6.49; N, 3.33.

4.1.4. *N*-[9-Fluorenylmethoxy(*n*-butyl)phosphinyl]-L-glutamic acid dimethyl ester (11). Chromatography conditions: ethyl acetate/hexane 4:1 v:v, R_f = 0.28. Yield: 33%. ^{1}H NMR (CD_3OD): δ 0.89 (t, J = 7.2 Hz, 3H), 1.30–1.51 (m, 4H), 1.65–1.88 (m, 3H), 2.04–2.11 (m, 1H), 2.38–2.44 (m, 2H), 3.62 and 3.66 (s, 3H), 3.68 (s, 3H), 3.83–3.92 (m, 1H), 4.18–4.39 (m, 3H), 7.30–7.43 (m, 4H), 7.67 (t, J = 6.9 Hz, 2H), 7.81 (d, J = 7.5 Hz, 2H). ^{31}P NMR (CD_3OD): δ 38.31, 39.08. Anal. Calcd for $C_{25}H_{32}NO_6P$: C, 63.3; H, 6.8; N, 3.14. Found: C, 63.37; H, 6.83; N, 2.95.

4.1.5. *N*-[9-Fluorenylmethoxy(*n*-butyl)phosphinyl]-D-glutamic acid dimethyl ester (11′). Compound **11′** was prepared from D-glutamic acid dimethyl ester. The NMR spectra of **11′** were identical to that of **11** above.

4.1.6. *N*-[9-Fluorenylmethoxy(phenyl)phosphinyl]-L-glutamic acid dimethyl ester (12). Chromatography conditions: ethyl acetate/hexane 2:1 v:v, $R_f = 0.23$. Yield: 36%. ^{1}H NMR (CD_3OD): δ 1.78–1.88 (m, 1H), 1.98–2.05 (m, 1H), 2.29–2.36 (m, 2H), 3.55 and 3.59 (s, 3H), 3.60 and 3.61 (s, 3H), 3.74–3.88 (m, 1H), 4.24–4.47 (m, 3H), 7.27–7.74 (m, 11H), 7.82 (d, $J = 7.5$ Hz, 2H). ^{31}P NMR (CD_3OD): δ 24.597, 25.299. Anal. Calcd for $C_{27}H_{28}NO_6P$: C, 65.7; H, 5.7; N, 2.8. Found: C, 65.57; H, 5.73; N, 2.80.

4.1.7. *N*-[9-Fluorenylmethoxy(phenyl)phosphinyl]-L-glutamic acid dimethyl ester (12′). Compound **12′** was prepared from D-glutamic acid dimethyl ester. The NMR spectra of **12′** were identical to that of **12** above.

4.1.8. General procedure for phosphonamidoic acids 1–3. Phosphonamidates **10–12** (0.202 mmol) were each dissolved in methanol (1.06 mL) to which an aqueous solution of LiOH (1 M, 1.013 mmol) was added. The reaction mixture was stirred 14 h, filtered, and concentrated in vacuo to dryness. The resulting solid was suspended in anhydrous methanol, filtered (0.2 μm Teflon membrane), and concentrated in vacuo to yield the respective trilithium salts.

4.1.9. *N*-[Hydroxy(ethyl)phosphinyl]-L-glutamic acid trilithium salt (1). Yield: 60%. ^{1}H NMR (CD_3OD): δ 1.03 (dt, $J = 18.0$, 7.5 Hz, 3H), 1.40–1.53 (m, 2H), 1.80–1.98 (m, 2H), 2.19–2.31 (m, 2H), 3.47–3.54 (m, 1H). ^{13}C NMR (CD_3OD): δ 8.02, 8.11, 22.11, 23.74, 33.54, 33.59, 35.08, 57.72, 183.17, 184.05. ^{31}P NMR (CD_3OD): δ 29.12.

4.1.10. *N*-[Hydroxy(*n*-butyl)phosphinyl]-L-glutamic acid trilithium salt (2). Yield: 75%. ^{1}H NMR (D_2O): δ 0.79 (t, $J = 7.2$ Hz, 3H), 1.23–1.45 (m, 6H), 1.70–1.75 (m, 2H), 2.11–2.16 (m, 2H), 3.35–3.38 (m, 1H). ^{13}C NMR (D_2O): δ 24.00, 25.48, 25.54, 28.26, 29.88, 32.72, 32.78, 56.89, 182.35, 183.43. ^{31}P NMR (CD_3OD): δ 27.87. FAB-HRMS $(M - Li)^-$ calcd 278.0955, found 278.0958 for $C_9H_{14}Li_3NO_6P$.

4.1.11. *N*-[Hydroxy(*n*-butyl)phosphinyl]-D-glutamic acid trilithium salt (2′). Compound **2′** was prepared from **11′**. The ^{1}H, ^{13}C, ^{31}P NMR spectra of **2′** were identical to that of **2** above.

4.1.12. *N*-[Hydroxy(phenyl)phosphinyl]-L-glutamic acid trilithium salt (3). Yield: 75%. ^{1}H NMR (D_2O): δ 1.77–1.86 (m, 2H), 2.09–2.19 (m, 2H), 3.39–3.46 (m, 1H), 7.27–7.28 (m, 3H), 7.67–7.70 (m, 2H). ^{13}C NMR (D_2O): δ 32.48, 34.01, 56.72, 128.30, 128.47, 130.38, 130.77, 130.89, 181.58, 183.35. ^{31}P NMR (CD_3OD): δ 12.04. FAB-HRMS $(M - Li)^-$ calcd 298.0642, found 298.0645 for $C_{11}H_{11}Li_3NO_6P$.

4.1.13. *N*-[Hydroxy(phenyl)phosphinyl]-D-glutamic acid trilithium salt (3′). Compound **3′** was prepared from **12′**. The ^{1}H, ^{13}C, ^{31}P NMR spectra of **3′** were identical to that of **3** above.

4.1.14. General procedure for phosphonamidothiolates 13–15. A solution of ethanedithiol monoacetate (6.0 mmol, 0.816 g) and DIPEA (6.0 mmol, 0.775 g) in benzene (22 mL) was added dropwise to a stirring solution containing alkylphosphonic dichloride (6.6 mmol), 1*H*-tetrazole (1.2 mmol, 0.084 g), and benzene (22 mL) under $Ar_{(g)}$ at 4 °C. The solution was stirred for 3 h during which it warmed to room temperature. A solution of neutralized L-glutamic acid dimethyl ester (7.2 mmol, 1.261 g), DIPEA (7.2 mmol, 0.931 g), and benzene (15.0 mL) was added and stirred for an additional 3 h. The reaction mixture was filtered, concentrated in vacuo to a yellow oil, and then purified by flash chromatography. Phosphonamidothiolate **13** was further separated chromatographically (ethyl acetate/hexane 10:1 v:v, $R_f = 0.25$ and 0.34) into the individual stereoisomers **13a** and **13b** to ultimately provide **4a** and **4b**, respectively.

4.1.15. (R_P)-*N*-[β-(Acetylmercapto)ethylthio(ethyl)phosphinyl]-L-glutamic acid dimethyl ester (13a). Yield: 36%. ^{1}H NMR ($CDCl_3$): δ 1.16–1.28 (m, 3H), 1.92–2.20 (m, 4H), 2.34 (s, 3H), 2.40–2.51 (m, 2H), 2.92–2.99 (m, 2H), 3.14–3.20 (m, 2H), 3.68 (s, 3H), 3.76 (s, 3H), 4.00–4.13 (m, 1H). ^{31}P NMR ($CDCl_3$): δ 53.58. Anal. Calcd for $C_{13}H_{24}NO_6PS_2$: C, 40.5; H, 6.3; N, 3.6. Found: C, 41.35; H, 6.32; N, 3.60.

4.1.16. (S_P)-*N*-[β-(Acetylmercapto)ethylthio(ethyl)phosphinyl]-L-glutamic acid dimethyl ester (13b). Yield: 36%. ^{1}H NMR ($CDCl_3$): δ 1.17–1.30 (m, 3H), 1.92–2.19 (m, 4H), 2.33 (s, 3H), 2.44–2.49 (m, 2H), 2.93–2.98 (m, 2H), 3.10–3.15 (m, 2H), 3.65 (s, 3H), 3.76 (s, 3H), 4.07–4.18 (m, 1H). ^{31}P NMR ($CDCl_3$): δ 52.68. Anal. Calcd for $C_{13}H_{24}NO_6PS_2$: C, 40.5; H, 6.3; N, 3.6. Found: C, 40.04; H, 6.28; N, 3.60.

4.1.17. *N*-[β-(Acetylmercapto)ethylthio(*n*-butyl)phosphinyl]-L-glutamic acid dimethyl ester (14). Chromatography conditions: ethyl acetate/hexane 10:1 v:v, $R_f = 0.26$. Yield: 29%. ^{1}H NMR ($CDCl_3$): δ 0.91–0.97 (m, 3H), 1.38–1.50 (m, 2H), 1.59–1.70 (m, 2H), 1.91–2.04 (m, 3H), 2.10–2.22 (m, 1H), 2.34 (s, 3H), 2.39–2.46 (m, 2H), 2.89–3.03 (m, 2H), 3.12–3.20 (m, 2H), 3.48–3.61 (m, 1H), 3.67 and 3.69 (s, 3H), 3.76 and 3.77 (s, 3H), 4.03–4.15 (m, 1H). ^{31}P NMR ($CDCl_3$): δ 50.99, 51.36.

4.1.18. *N*-[β-(Acetylmercapto)ethylthio(*n*-butyl)phosphinyl]-D-glutamic acid dimethyl ester (14′). Compound **14′** was prepared from D-glutamic acid dimethyl ester. The NMR spectra of **14′** were identical to that of **14** above.

4.1.19. *N*-[β-(Acetylmercapto)ethylthio(phenyl)phosphinyl]-L-glutamic acid dimethyl ester (15). Chromatography conditions: ethyl acetate/hexane 4:1 v:v, $R_f = 0.22$. Yield: 19%. ^{1}H NMR ($CDCl_3$): δ 1.92–2.04 (m, 1H),

2.09–2.21 (m, 1H), 2.29 (s, 3H), 2.43–2.50 (m, 2H), 2.88–3.01 (m, 2H), 3.05–3.11 (m, 2H), 3.62 and 3.67 (s, 3H), 3.70 and 3.77 (s, 3H), 3.99–4.19 (m, 1H), 7.45–7.59 (m, 3H), 7.83–7.93 (m, 2H). ^{31}P NMR ($CDCl_3$): δ 32.027, 32.942.

4.1.20. *N*-[β-(Acetylmercapto)ethylthio(phenyl)phosphinyl]-D-glutamic acid dimethyl ester (15′). Compound **15′** was prepared from D-glutamic acid dimethyl ester. The NMR spectra of **15′** were identical to that of **15** above.

4.1.21. General procedure for phosphonamidothioic acids 4a and 4b. A single isomer of phosphonamidothiolate **13** (0.155 mmol, 0.060 g) and L-(−)-α-methylbenzylamine (0.326 mmol, 0.040 g) was refluxed in methanol (3.0 mL) 13 h followed by concentration in vacuo to give a yellow oil. The resulting crude product was dissolved in water and washed with a diethyl ether/hexane mixture (1:1, v:v). The aqueous layer was concentrated in vacuo to yield the intermediate methylbenzylammonium phosphonamidothioate (0.141 mmol, 0.057 g), which was subsequently dissolved in methanol (0.71 mL) and stirred 14 h with an aqueous solution of LiOH (1 M, 0.71 mL). The reaction mixture was concentrated in vacuo to dryness, suspended in anhydrous methanol, filtered (0.2 μm Teflon membrane), and concentrated in vacuo to yield the trilithium salts **4a** and **4b**.

4.1.22. (R_P)-*N*-[Hydroxy(ethyl)phosphinothioyl]-L-glutamic acid trilithium salt (4a). Yield: 86%. ^{1}H NMR (D_2O): δ 0.93–1.07 (m, 3H), 1.64–1.92 (m, 4H), 2.11–2.17 (m, 2H), 3.42–3.57 (m, 1H). ^{13}C NMR (D_2O): δ 7.75, 7.81, 30.09, 31.35, 32.32, 32.38, 34.17, 57.14, 182.04, 183.30. ^{31}P NMR (D_2O): δ 71.01.

4.1.23. (S_P)-*N*-[Hydroxy(ethyl)phosphinothioyl]-L-glutamic acid trilithium salt (4b). Yield, 86%. ^{1}H NMR (D_2O): δ 0.92–1.04 (m, 3H), 1.63–1.94 (m, 4H), 2.11–2.17 (m, 2H), 3.48–3.61 (m, 1H). ^{13}C NMR (D_2O): δ 7.92, 7.97, 29.25, 30.51, 32.45, 32.51, 34.28, 57.08, 182.09, 183.29. ^{31}P NMR (D_2O): δ 69.56.

4.1.24. General procedure for phosphonamidodithionates 16–18. Diastereomeric mixtures of phosphonamidothiolates **13**–**15** (0.451 mmol) each were refluxed 2.5 h in toluene (7.0 mL) with Lawesson's reagent (0.248 mmol, 0.100 g). The reaction mixture was concentrated in vacuo to a yellow oil, which was purified by flash chromatography.

4.1.25. *N*-[β-(Acetylmercapto)ethylthio(ethyl)phosphinothioyl]-L-glutamic acid dimethyl ester (16). Chromatography conditions: CH_2Cl_2/ethyl acetate 100:3 v:v, R_f = 0.35. Yield: 70%. ^{1}H NMR ($CDCl_3$): δ 1.24–1.32 (m, 3H), 1.90–2.20 (m, 4H), 2.34 (s, 3H), 2.40–2.47 (m, 2H), 3.00–3.22 (m, 4H), 3.37–3.50 (m, 1H), 3.68 and 3.69 (s, 3H), 3.76 (s, 3H), 3.87–4.27 (m, 1H). ^{31}P NMR ($CDCl_3$): δ 89.43, 90.82. Anal. Calcd for $C_{13}H_{24}NO_5PS_3$: C, 38.9; H, 6.0; N, 3.5. Found: C, 39.14; H, 5.91; N, 3.37.

4.1.26. *N*-[β-(Acetylmercapto)ethylthio(*n*-butyl)phosphinothioyl]-L-glutamic acid dimethyl ester (17). Chromatography conditions: CH_2Cl_2/ethyl acetate 50:1 v:v, R_f = 0.24. Yield: 76%. ^{1}H NMR ($CDCl_3$): δ 0.58 (t, J = 7.2 Hz, 3H), 1.40–1.50 (m, 2H), 1.62–1.72 (m, 2H), 1.89–2.20 (m, 4H), 2.34 (s, 3H), 2.39–2.49 (m, 2H), 2.94–3.16 (m, 4H), 3.35–3.51 (m, 1H), 3.68 and 3.68 (s, 3H), 3.76 (s, 3H), 4.06–4.26 (m, 1H). ^{31}P NMR ($CDCl_3$): δ 81.05, 82.45. Anal. Calcd for $C_{15}H_{28}NO_5PS_3$: C, 41.9; H, 6.6; N, 3.3. Found: C, 41.97; H, 6.54; N, 3.27.

4.1.27. *N*-[β-(Acetylmercapto)ethylthio(*n*-butyl)phosphinothioyl]-D-glutamic acid dimethyl ester (17′). Compound **17′** was prepared from **14′**. The NMR spectra of **17′** were identical to that of **17** above.

4.1.28. *N*-[β-(Acetylmercapto)ethylthio(phenyl)phosphinothioyl]-L-glutamic acid dimethyl ester (18). Chromatography conditions: ethyl acetate/hexane 4:1 v:v, R_f = 0.31. Yield: 74%. ^{1}H NMR ($CDCl_3$): δ 1.99–2.03 (m, 1H), 2.08–2.12 (m, 1H), 2.30 (s, 3H), 2.38–2.47 (m, 2H), 2.99–3.10 (m, 4H), 3.61 and 3.67 (s, 3H), 3.70 and 3.75 (s, 3H), 4.09–4.12 (m, 1H), 7.45–7.56 (m, 3H), 8.01 (q, J = 7.5 Hz, 2H). ^{31}P NMR ($CDCl_3$): δ 72.51, 74.15. Anal. Calcd for $C_{17}H_{24}NO_5PS_3$: C, 45.4; H, 5.4; N, 3.1. Found: C, 45.34; H, 5.28; N, 3.11.

4.1.29. *N*-[β-(Acetylmercapto)ethylthio(phenyl)phosphinothioyl]-D-glutamic acid dimethyl ester (18′). Compound **18′** was prepared from **15′**. The NMR spectra of **18′** were identical to that of **18** above.

4.1.30. General procedure for phosphonamidodithioic acids 7–9. Phosphonamidodithionates **16**–**18** (0.114 mmol) were each dissolved in methanol (0.188 mL) and reacted with a 7 N methanolic solution of NH_3 (0.482 mL) without stirring under an $Ar_{(g)}$ atmosphere for 45 min. The product mixtures were immediately concentrated in vacuo, washed with diethyl ether, and filtered to yield white solids. Each solid was dissolved in methanol (0.385 mL) and stirred 14 h with an aqueous 1 M LiOH solution (0.385 mL). The reaction mixture was concentrated in vacuo to dryness, suspended in anhydrous methanol, and filtered (0.2 μm Teflon membrane) to, respectively, give phosphonamidodithioic acids **7**–**9** as the trilithium salts.

4.1.31. *N*-[Hydroxy(ethyl)phosphinodithioyl]-L-glutamic acid trilithium salt (7). Yield: 31%. ^{1}H NMR (CD_3OD): δ 0.71–0.78 (m, 3H), 1.42–1.57 (m, 4H), 1.71–1.92 (m, 2H), 3.35–3.44 (m, 1H). ^{13}C NMR (CD_3OD): δ 9.43, 33.97, 35.51, 39.19, 40.16, 59.07, 183.13, 183.46. ^{31}P NMR (D_2O): δ 90.54. FAB-HRMS $(M - Li)^-$ calcd 282.0185, found: 282.0187 for $C_7H_{11}Li_3NO_4PS_2$.

4.1.32. *N*-[Hydroxy(*n*-butyl)phosphinodithioyl]-L-glutamic acid trilithium salt (8). Yield: 36%. ^{1}H NMR (CD_3OD): δ 0.96 (t, J = 7.5 Hz, 3H), 1.41–1.50 (m, 2H), 1.61–1.72 (m, 2H), 1.88–1.97 (m, 2H), 2.05–2.13 (m, 2H), 2.27–2.32 (m, 2H), 3.72–3.81 (m, 1H). ^{13}C NMR (D_2O): δ 13.17, 13.38, 23.00, 23.25, 26.39, 32.09, 34.10, 43.49, 44.42, 57.63, 181.75, 181.16. ^{31}P NMR

(CD_3OD): δ 86.17. FAB-HRMS $(M - Li)^-$ calcd 310.0498, found: 310.0501 for $C_9H_{15}Li_3NO_4PS_2$.

4.1.33. *N*-[Hydroxy(*n*-butyl)phosphinodithioyl]-D-glutamic acid trilithium salt (8′). Compound **8′** was prepared from **17′**. The NMR spectra of **8′** were identical to that of **8** above.

4.1.34. *N*-[Hydroxy(phenyl)phosphinodithioyl]-L-glutamic acid trilithium salt (9). Yield: 67%. ^1H NMR (CD_3OD): δ 1.78–1.90 (m, 4H), 3.69–3.77 (m, 1H), 7.21–7.27 (m, 3H), 8.08–8.15 (m, 2H). ^{13}C NMR (CD_3OD): δ 33.55, 35.35, 59.12, 128.72, 128.35, 130.12, 131.42, 131.57, 181.86, 183.62. ^{31}P NMR (CD_3OD): δ 74.43. FAB-HRMS $(M - Li)^-$ calcd 330.0185, found: 330.0188 for $C_{11}H_{11}Li_3NO_4PS_2$.

4.1.35. *N*-[Hydroxy(phenyl)phosphinodithioyl]-D-glutamic acid trilithium salt (9′). Compound **9′** was prepared from **18′**. The NMR spectra of **9′** were identical to that of **9** above.

4.2. Stability studies of inhibitors

Inhibitors **1**–**3**, **4a**–**6a**, **4b**–**6b**, and **7**–**9** (10 μmol) were each dissolved in 1 mL of Tris buffer (100 mM, pH 7.3) to give a 10 mM inhibitor solution and 0.5 mL of the resulting solution was transferred to a 5 mm NMR tube. ^{31}P NMR data were acquired every 10–20 min for up to 10 h from the time when the buffer was added.

4.3. Enzyme inhibition

4.3.1. General. Glutamate carboxypeptidase (carboxypeptidase G from *Pseudomonas* sp. strain ATCC 25301, C 4053) and methotrexate were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest purity and purchased from commercial sources.

4.3.2. Inhibition assay procedures. Inhibition of glutamate carboxypeptidase by the synthetic inhibitors was determined as described previously.[6] Briefly, a typical incubation mixture (final volume 0.25 mL) was prepared by the addition of 200 μL Tris buffer (50 mM, pH 7.3) to either a 25 μL mixture of both methotrexate and inhibitor in buffer or 25 μL of a buffered solution of methotrexate alone. The enzymatic reaction was initiated by the addition of 25 μL of an enzyme solution (0.17–0.21 μg protein/mL buffer). In all cases, the final concentration of methotrexate was 10 μM while the inhibitor concentration varied from 7.5 to 120 μM. The reaction was allowed to proceed for 1 min with constant shaking at 30 °C and was terminated by the addition of 100 μL methanolic TFA (1% trifluoroacetic acid by volume in methanol) followed by vortexing and centrifugation (7000*g*). A 100 μL aliquot of the resulting supernatant was then quantified by subsequently quantified by HPLC. Methotrexate and its hydrolytic product (4-(*N*-[2,4-diamino-6-pteridinylmethyl]-*N*-methylamino)benzoic acid) were separated and quantified with an analytical reversed phase HPLC column (4.6 × 150 mm, Sphereclone 5 u ODS(2), Phenomenex, Torrence, CA) with a mobile phase of CH_3OH/(potassium phosphate, 50 mM, pH 6.8) (22:78, v:v). At a flow rate of 0.9 mL/min, methotrexate and its hydrolytic product were detected at 304 nm with retention times of 3.8 and 7.7 min, respectively. Under the assay conditions described above, it was noted that the initial substrate concentration was not substantially depleted during the time course of the incubation (e.g., approximately 10% conversion to product was observed for incubations with the lowest substrate concentration, 1 μM).

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References and notes

1. Robinson, M. B.; Blakely, R. D.; Couto, R.; Coyle, J. T. *J. Biol. Chem.* **1987**, *262*, 14498.
2. Carter, R. E.; Feldman, A. R.; Coyle, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 749.
3. Reisenauer, A. M.; Krumdieck, C. L.; Halsted, C. H. *Science* **1977**, *198*, 196.
4. (a) Goldman, P.; Levy, C. C. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 1299; (b) Levy, C. C.; Goldman, P. *J. Biol. Chem.* **1967**, *242*, 2933; (c) McCullough, J. L.; Chabner, B. A.; Bertino, J. R. *J. Biol. Chem.* **1971**, *246*, 7207; (d) Sherwood, R. F.; Melton, R. G.; Alwan, S. M.; Hughes, P. *Eur. J. Biochem.* **1985**, *148*, 447.
5. Kahn, T. H.; Eno-Amooquaye, E. A.; Searle, F.; Browne, P. J.; Osborn, H. M. I.; Burke, P. J. *J. Med. Chem.* **1999**, *42*, 951.
6. (a) Rodriguez, C. E.; Lu, H.; Dinh, T. T.; Mlodnosky, K. L.; Dastgah, A.; Lam, V. Q.; Nichols, C. B.; Berkman, C. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1415; (b) Lu, H.; Berkman, C. E. *Bioorg. Med. Chem.* **2001**, *9*, 395; (c) Rodriguez, C. E.; Lu, H.; Martinez, A. R.; Brunelle, A.; Berkman, C. E. *J. Enzym. Inhib.* **2001**, *16*, 359.
7. (a) Bell, C. F. In *Principles and Applications of Metal Chelation*; Atkins, P. W., Holker, J. S. E., Holliday, A. K., Eds.; Oxford University Press: Oxford, 1977; (b) Prasad, A. S. In *Biochemistry of Zinc*; Frieden, E., Ed.; Plenum: New York, 1993.
8. Lu, H.; Hu, Y.; Choy, C. J.; Mallari, J. P.; Villanueva, A. F.; Arrozal, A. F.; Berkman, C. E. *Tetrahedron Lett.* **2001**, *42*, 4313.
9. Watanabe, Y.; Nakatomi, M. *Tetrahedron Lett.* **1998**, *39*, 1583; Seeberger, P. H.; Yau, E.; Caruthers, M. H. *J. Am. Chem. Soc.* **1995**, *117*, 1472.
10. Hall, C. R.; Inch, T. D. *Phosphorus Sulfur* **1979**, *7*, 171.
11. Compound information: exptl crystal color = colorless; chemical melting point = 383–385 °C; exptl crystal description = prismatic; exptl crystal preparation = acetone–hexane. Unit cell information: cell length a = 9.729; cell length b = 14.694; cell length c = 17.600; cell angle α = 90.00; cell angle β = 90.00; cell angle γ = 90.00; cell volume = 2516.0; symmetry cell setting = orthorhombic; symmetry space group name H-M = *P* 21 21 21; cell formula units Z = *4*; exptl crystal density = 1.250.

12. Jackson, J. A.; Berkman, C. E.; Thompson, C. M. *Tetrahedron Lett.* **1992**, *41*, 6061.
13. Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1982**, *105*, 1619–1626.
14. (R_P) and (S_P) refer to the stereochemical configuration of phosphorus.
15. Lu, H.; Ng, R.; Shieh, C. C.; Martinez, A. R.; Berkman, C. E. *Phosphorus, Sulfur Silicon* **2003**, *178*, 17.
16. The correlation coefficient for the Dixon analysis of compounds **4b** and **8** were both 0.97.
17. Hill, J. M.; Lowe, G. (1995) *J. Chem. Soc., Perkin Trans. 1* **2001**.